REMARKS

Claim Amendments

Claims 17, 19-21, 23-24, 28-29, 31, 33-34, 38-39, 41, 43, 46, 49 and 54-56 are currently pending in the application. Claims 39, 46, 54 and 55 are newly cancelled without prejudice to Applicant's rights to pursue the subject matter of these claims in another application. Claims 57 through 61 are newly added. Claims 17, 19-21, 23-24, 28-29, 31, 33-34, 38, 41, 43, 49, and 56 are newly amended. The claim amendments find support in the specification and are discussed below.

Independent claim 17 has been amended so as to limit the oligonucleotide to one which is "*specific only for RNA, and/or cDNA complementary to said RNA*", in accordance with the oligonucleotides (i.e. primers) claimed in previously/instantly pending claim 19 and previously pending claim 55.

Independent claim 19 has been amended to clarify that the primers are specific only for RNA (and/or cDNA complementary to said RNA) encoded by said gene in said sample as was noted in currently cancelled claim 54. Claim 19 has also been amended to clarify that it is the control RNA which has been detected in said samples. Support for these amendment is found throughout the published application (US 2007/0031841; hereinafter "the Published Application").

Claims 20, 28 and 41, depending from claim 17 or 57; and claim 38, depending from claim 19 or 58, have each been amended for increased clarity so as to recite "RNA encoded by said gene of step (a)".

Claim 43 has been amended so as to limit the disease to only colorectal cancer, and the limitation of claim 43 where the disease is limited to diabetes or heart failure has been incorporated into new claim 59 or 60, respectively.

New claims 57 and 58 have been added, essentially corresponding to claims 17 and 19, respectively, but with the recitation "*blood samples which have not been fractionated into cell types*" of claims 17 and 19 being modified so as to recite "*blood samples which comprise leukocytes which have not been fractionated into cell types*". Claims 57 and 58 are added in

order to more clearly distinguish the claimed invention over the prior art, i.e. by clarifying that the RNA analyzed in the claimed methods is of blood samples which include all of the types of leukocytes of whole blood, i.e. RNA of blood samples which include granulocytes, the majority cellular component of leukocytes, in addition to mononuclear cells (T-lymphocytes, B-lymphocytes and monocytes). Applicant's instant claiming of "blood samples which comprise leukocytes which have not been fractionated into cell types" finds clear support in the specification, including at Figure 5C which shows standardized levels of insulin gene in different fractions of leukocytes (i.e. in granulocytes, T-lymphocytes, B-lymphocytes and monocytes labeled "G.R.", "CD 3+", "CD19" and "MONO", i.e., respectively) which collectively constitute whole leukocytes or "leukocytes which have not been fractionated into cell types", as recited in the claims. ,. It is well known to the ordinarily skilled artisan that CD3 and CD19 are specific cell surface markers of T-lymphocytes and B-lymphocytes (refer, for example, to the enclosed Abstract of Casey et al., 1988. A simplified plastic embedding and immunohistologic technique for immunophenotypic analysis of human hematopoietic and lymphoid tissues. Am J Pathol. 131:183-9). The fact that granulocytes (G.R.), lymphocytes [T-lymphocytes (CD 3+) and B-lymphocytes (CD19+)] and monocytes (MONO) collectively represent all of the types of leukocytes found in blood is evident in Immunobiology. Garland Publishing. 2001. Fifth Edition. Janeway, Travers, Walport, and Shlomchik, eds. Fig. A.23, of record., which clearly teaches that leukocytes are composed of granulocytes and mononuclear cells, and that the latter are composed of lymphocytes and monocytes. Additional support for the term "leukocytes" is found at paragraph [0005] of the Published Application.

New claim 61, depending from claim 17, 19, 57 or 58, has been added, limiting said gene to one which is predominantly expressed in said non-blood tissue. Specification support for this limitation can be found, for example, at paragraph [0008] of the published application.

35 U.S.C. § 112, 2nd Paragraph Rejections – Indefiniteness

The Examiner has rejected claims 19, 21, 24, 29, 31, 33, 34, 38, 39, 43, 46, 49, 55 and 56 under 35 U.S.C. § 112, second paragraph, as being indefinite over the recitation "RNA of unfractionated cells of lysed blood samples". Applicant wishes to point out that claim 19 does not include this recitation.

Solely in order to expedite prosecution in this case, Applicant has cancelled claims 55 and 56, rendering moot the rejections of these claims, and to amended current claims 21, 24, 29, 31, 33, 34, 38, 39, 43, 46, and 49 so as to remove references to claim 55 and 56. Applicant retains the right, however, to pursue the subject matter of the cancelled claims in another application. In view of the above amendments and arguments, Applicant respectfully requests withdrawal of the rejections.

35 U.S.C. § 112, 1st Paragraph Rejections – Written Description

The Examiner has rejected claims 39 and 46 as failing to comply with the written description requirement. More particularly the Examiner has objected to the language "subjects having said disease..._have no overt symptoms with respect to said disease" found in claim 39, and the language "said subjects having said disease are asymptomatic with respect to said disease" found in claim 46. The Examiner also argues that the phrase "subjects having said disease are asymptomatic with respect to said disease" is new matter.

Applicant disagrees the phrase "subjects having said disease are asymptomatic with respect to said disease" is new matter, and further disagrees that there is no written support for claims 39 and 46, however in order to expedite prosecution, Applicant has cancelled claims 39 and 46 subject to the right to pursue the subject matter of these claims in another application.

In view of the above amendments, Applicant respectfully requests withdrawal of the rejections.

35 U.S.C. § 102 Rejections – Anticipation

Claims 17, 19-21, 23-24, 28-29, 31, 33-34, 38, 39, 41, 46, 49, 54, 55 and 56 are rejected as being anticipated by WO 98/24935 to Ralph et al. under 35 U.S.C. 102(b) and 102(a) and US 6,190,857 to Ralph et al. pursuant to 35 U.S.C. 102(e). More particularly, the Examiner asserts that these references have substantially identical disclosures, and that each of these references teaches each of the elements of the claimed invention.

Applicant respectfully traverses the rejections with regard to both WO 98/24935 to Ralph et al. and to US 6,190,857 to Ralph et al.. Anticipation requires that the purported prior art reference disclose each and every limitation of the claim. *Atlas Powder Company et al. v.*

IRECO, Incorporated et al., 190 F.3d 1342, 1347 (Fed. Cir. 1999). Applicant contends that Ralph *et al.* does not teach each and every limitation of the instant claims.

The Examiner specifically contends, as an essential basis of the rejections, that U.S. Pat. No. 6,190,857 to Ralph *et al.* (hereinafter for purposes of this Amendment and Reply unless otherwise stated "Ralph *et al.*") teaches at Col. 98, lines 5-6 (section entitled 5.6.2 "*Relative Quantitative RT-PCR*") detection of a presence of disease marker RNA in "DNA-free total RNA from peripheral blood", and that such "DNA-free total RNA from peripheral blood" is "RNA of blood samples which have not been fractionated into cell types", as required by the claims. However, Applicant respectfully submits, as clearly demonstrated and attested to in the attached expert declaration dated August 18, 2007 (refer, for example, to paragraphs #14-18 of the declaration), and as clearly demonstrated by Applicant hereinbelow, that the cited statement phrase "*DNA-free total RNA from peripheral blood" of Ralph et al. unambiguously* refers only to RNA from **isolated mononuclear cells**, and hence can not be interpreted as "RNA of blood samples which have not been fractionated into cell types", as required by the claims. As such, Ralph *et al.* clearly cannot be interpreted as teaching detection of the presence of disease marker RNA in "RNA of blood samples which have not been fractionated into cell types", as required by the claims. Thus, Applicant respectfully submits that Ralph *et al.* fails to each of the elements of the claimed invention, including the element "RNA of blood samples which have not been fractionated into cell types" as required by independent claims 17 and 19.

In clear support of Applicant's position, expert declarant, Paul Dobner, Ph.D., states unequivocally in the attached expert declaration dated August 18, 2007:

> "*RNA of blood samples which have not been fractionated into cell types" or obtained from "lysis of unfractionated cells" is not described in Ralph et al. as stated by the Examiner*", (paragraph #13),

The expert declarant further states in paragraph #13 that, in contrast, the examples from Ralph *et al.* cited by the Examiner all use RNA prepared from the isolated mononuclear cell fraction of blood. Thus, Applicant respectfully submits that Ralph et al. only teaches the use of blood samples which have been fractionated into cell types, and in particular teaches using a fraction of leukocytes, and as such does not anticipate the claims. In particular, as attested to in the attached expert declaration (see, for example, paragraphs #16-21), Example 5.6.2, which includes the phrase "DNA-free total RNA from peripheral blood" cited by the Examiner, clearly

refers to RNA obtained from a cell fraction of leukocytes, namely from isolated mononuclear cells prepared using Ficoll density gradient fractionation, in accordance with section 4.9.1 (Col. 62) of Ralph *et al.* As can be clearly understood due to its being the introduction to the RNA-related experimental protocols disclosed in Ralph *et al.*, and due to explicit indications as elaborated below and in the enclosed expert declaration, section 4.9.1 of Ralph *et al.* is the master protocol which is followed in each of the examples disclosed by Ralph *et al.* for isolation of blood cells for RNA analysis.

Applicant respectfully submits that it can be clearly demonstrated, as follows, that Ralph *et al.* teaches the use of RNA obtained only from an isolated mononuclear cell fraction of blood. The passage cited by the Examiner in section 5.6.2, which describes the relative quantitative RT-PCR performed in section 5.6/Example 6 ("*Two mRNAs with Conserved Sequence Motifs are up Regulated in Nucleated Blood Cells of Patients with Metastatic Breast or Prostate Cancer*"), specifically cite that "DNA-free total RNA from the peripheral blood of healthy volunteers or patients with either metastatic prostate or breast cancer were analyzed by relative quantitative RT-PCR as described in section 4.11.3" (column 98, line 8).

Section 4.11.3 is the "*Relative Quantitative RT-PCR*" protocol section of 4.11 (entitled "*Detection and Diagnosis of Metastatic Breast and Prostate Cancer*"), which describes under section 4.11.1 (entitled "*Preparation of RNA*") that "RNA was prepared from nucleated circulating peripheral blood cells as described in Section 4.9.1 above" (column 67 line 12-13), i.e. from the mononuclear cell fraction of blood", as demonstrated below. Section 4.11.1 (2nd paragraph) clearly teaches use of RNA prepared according to section 4.9.1, i.e. of RNA prepared from an isolated mononuclear cell fraction of blood, for performing relative quantitative RT-PCR, the technique employed in the passages cited by the Examiner at section 5.6.2 (entitled "*Relative Quantitative RT-PCR*").

Additionally section 5.6/Example 6, which includes the passages cited by the Examiner, dictates that the RNA fingerprinting is performed as described in section 4.12 (see column 95, line 37). Section 4.12 describes the use of RNA prepared as described in section 4.11.1 and RNA fingerprinting performed as described in section 4.11.2 (column 70, lines 8-11).

Thus, section 5.6/Example 6, which includes the passages in section 5.6.2 cited by the Examiner, describes experiments performed using the methods taught in section 4.11 including the preparation of RNA from nucleated circulating peripheral blood cells as described in section

4.11.1, using the procedure of 4.9.1, RNA fingerprinting as described in 4.11.2 and the RT-PCR as described in 4.11.3.

Section 4.9.1 (starting at column 62), describes the preparation of blood cells for RNA isolation as follows:

> "*Blood was drawn from cancer patients and normal individuals into Vacutainer CPT tubes with ficoll gradients (Becton Dickinson and Company, Frankin Lanes, N.J.). The tubes were centrifuged to separate the red blood cells from various types of nucleated cells, collectively referred to as the buffy coat, and from blood plasma. Total cell RNA was isolated from the buffy coats by the RNA STAT-60 method (Tel-Test, Inc., Friendswood, Tex.).*" (see column 62, lines 33-40).

As would be understood by a person skilled in the art, and attested to in paragraphs #18 and #19 of the attached expert declaration, Becton Dickinson Vacutainer™ CPT™ tubes are neither designed nor useful for collecting cells which haven't been fractionated into cell types. Rather, these tubes are specifically designed for the isolation and selective recovery of mononuclear cells from blood (see the BD Vacutainer™ CPT™ tube manual which accompanies both the 4ml draw capacity Vacutainer™ CPT™ tube (Ref 362760) and the 8ml draw capacity Vacutainer™ CPT™ tube (Ref 362761) (enclosed) (hereinafter the "CPT Tube Manual").

In fact, the CPT Tube Manual describes the application of the products at page 1 of the manual as follows:

> "*BD Vacutainer™ CPT™ Cell Preparation Tube with Sodium Citrate is an evacuated tube intended for the collection of whole blood and the separation of mononuclear cells. The cell separation medium is comprised of a polyester gel and a density gradient liquid.*"

As noted at p. 7 of the CPT Tube Manual at paragraph 5, "after centrifugation, mononuclear cells and platelets will be in a whitish layer just under the plasma layer (see Figure 2). Figure 2, also found at p.7, graphically demonstrates the results after centrifugation with the Vacutainer™ CPT™ tube. In particular, after centrifugation it is clear that the mononuclear cells form an easily recoverable layer above the surface of the dense Ficoll layer located above the polyester gel barrier, whereas the granulocytes are essentially inaccessibly sequestered at the bottom of the tube below the polyester gel barrier.

Thus, the only type of blood cells which can be isolated using the CPT™ Vacutainer™ tube are mononuclear cells, and it would be very clear to a person skilled in the art, upon reading

section 4.9.1 in the context of Ralph *et al.* and having knowledge of blood and RNA isolation procedures, that the preparation of RNA described by Ralph *et al.* is that of RNA obtained from an isolated cellular fraction of blood, and that the phrases "total cell RNA" and "RNA was prepared from nucleated circulating peripheral blood cells" in Ralph et al., refer to RNA of isolated mononuclear cells.

The fact that the same RNA is utilized for both RNA fingerprinting and relative quantitative RT-PCR is consistently taught throughout the specification.

Thus, as described above, after isolating RNA from mononuclear cells as described in section 4. 9.1, the RNA is DNase I treated and "one aliquot was set aside for relative quantitative RT-PCR confirmation using the external standard method" and "a second aliquot was used to fingerprint the RNA" (column 62, line 55-59). Similarly in the methods described in section 4.11, the RNA is prepared in accordance with fractionation method set out in section 4.9.1 and the RNA "selected for further analysis by RNA fingerprinting and relative quantitative RT-PCR" (column 67, line 45-46).

As attested to in the attached expert declaration, all of the teachings within Ralph et al. are consistent with isolation of RNA from the mononuclear cell fraction of blood. The teachings relating to identification of two or more markers useful for diagnosing prostate or breast cancer are outlined, in part, in section 4.11 entitled "Detection and Diagnosis of Metastatic Breast and Prostate Cancer". As previously noted, section 4.11.1 states "RNA was prepared from nucleated circulating peripheral blood cells as described in Section 4.9.1 above." (see column 67 line 12-13).

As discussed above section 4.9.1 clearly teaches the use of Ficoll gradient fractionation (via CPT Vacutainer tubes) to obtain mononuclear cells for RNA isolation. Section 4.11.1, continues to use the terminology of "nucleated peripheral blood cells" when referring to the RNA as isolated using 4.9.1, therefore, it is clear that Ralph et al. is actually referring to isolation of RNA from the mononuclear cell fraction blood when describing isolation of RNA from blood.

Section 4.12 describes the methods particularly used to identify the two genes UC331 and UC332 for diagnosis of metastatic breast and prostate cancer. Section 4.12.1 describes the use of 8 ml of peripheral blood (column 69, line 65-67), and section 4.12.2 refers to section 4.11.1 for a description of the preparation method used to obtain the RNA utilized in the experiments. As

noted above, section 4.11.1 refers to the mononuclear cells isolated as described in section 4.9.1 (column 67 line 12-13).

With respect to Ralph et al.'s use of the terms "leukocytes" and "peripheral blood leukocytes", it is clear from the context of this reference as a whole and from specific teachings therein, that these terms do not refer to leukocytes which are not fractionated into cell types, but rather refer only to a mononuclear cell fraction of leukocytes isolated using a Ficoll gradient via CPT™ Vacutainer™ tubes. For example, the Results section of section 5.6/Example 6 which, as described above and as demonstrated and attested to in the attached expert declaration, is performed using RNA from isolated mononuclear cells, clearly refers to isolated mononuclear cells as "leukocytes", in accordance with the recitation: "**In this Example**, RNA fingerprinting identified two cDNA fragments derived from mRNA species that had higher steady state abundances in the peripheral blood **leukocytes** of patients with recurrent metastatic prostate cancer as compared to a group of healthy volunteers." (Col. 98, lines 32-35). Additionally, Example 5 discusses the measurement of IL-8 mRNA using relative quantitative RT-PCR from "peripheral blood leukocytes" (see column 89, line 29-34; column 91, line 44-45). In summarizing the relative quantitative RT-PCR studies of this example, the use of mononuclear cells is particularly noted as follows: "As such, by examining the peripheral blood **mononuclear cell** population, evidence of cancer presence was obtained without requiring any knowledge of its physical location in the body" (column 94, line 19-22).

The attached expert declaration states:

> "*Although blood fractionated in this way consists principally of mononuclear cells, mainly B and T lymphocytes and monocytes as just discussed, Ralph et al. refer to this fraction in various ways including "nucleated circulating peripheral blood cells" (Col. 67, lines 12-13), "the buffy coat" (Col. 62, line 38), and "peripheral blood leukocytes" (Col. 98, line 34). Thus, these three terms are used equivalently by Ralph et al.*" (paragraph #19 of the declaration); and further states

> "*It should be noted that although Ralph et al. refer to "peripheral blood leukocytes", which is known in the art as a blanket term for all white blood cells, the detailed methods that they present in their working examples are for the isolation of mononuclear cells, including the T and B lymphocytes and monocytes, but largely excluding the more numerous granulocytes or polymorphonuclear cells. Thus, in my expert opinion, the term "peripheral blood leukocytes" as used in Ralph et al. clearly refers to the isolated mononuclear fraction*" (paragraph #22 of the declaration).

The only other reference to a method of purifying RNA from an isolated cell preparation is outlined in section 4.9.2. A careful reading of this section indicates that it merely teaches isolation of RNA from the sample using guanidinium thiocyanate phenol chloroform, rather than RNA STAT-60 as taught in section 4.9.1, and that it does not suggest that the sample from which RNA is isolated is anything other than a sample of a mononuclear cell fraction of blood as outlined in section 4.9.1. Section 4.9.2 notes "Total RNAs were isolated from peripheral blood samples as described (Chomczynski & Sacchi, 1987)". The Chomczynski & Sacchi reference (attached) is clearly cited for teaching a method of isolating RNA from a sample, and not for teaching how the sample itself is obtained prior to RNA extraction (see p.158, results and discussion section). Thus, the reference to this method, like reference in section 4.9.1. to the RNA STAT-60 method (see column 62, line 39) advises as to which RNA isolation method is to be used once the mononuclear cell layer is isolated from peripheral blood as described. The fact that Ralph et al. continues to refer to the RNA in this example as "total cell RNA" (see column 63, line 28) is consistent with the use of isolated mononuclear cells for RNA isolation. The use of the term "total RNAs" in section 4.9.2 is also fully consistent with that section describing RNA from isolated mononuclear cells, in accordance with the phrase "Total RNAs" being clearly used in section 4.11.1 to indicate RNA prepared from nucleated blood cells as described in section 4.9.1, i.e. prepared from isolated mononuclear cells. Namely, the paragraph starting at line 12 of Col. 67 of section 4.11.1 refers to RNA prepared as described in section 4.9.1 (lines 13-14; i.e. prepared from mononuclear cells) as "total RNAs" (line 19). Moreover, "Total RNA" is a well established term of art to refer to all of the species of RNA present in a given cell population including, for example, mRNA, rRNA and tRNA as opposed to referring only, for example, to mRNA (see for example Lepinske, Promega Notes Magazine No. 63 1997 p.17 discussing when to isolate total RNA in preference to mRNA). The term "total RNA" is also used in this same manner in the Chomczynski reference (see the Abstract, p.156).

Furthermore, Paul Dobner, Ph.D., expert in the art, states in his attached declaration, dated August 18, 2007:

> "*These considerations indicate that the method described in Section 4.9.2 involves the use of the method of Chomczynski and Sacchi to prepare RNA from mononuclear cells in accordance with the procedure outlined in section 4.9.1, even though this is not explicitly stated in Section 4.9.2. The statement in the method that "total RNAs were isolated from peripheral blood samples" cannot be interpreted as designating the isolation of RNA from whole blood, since the authors used similar terminology*

at various points in the text to refer to RNA prepared from isolated mononuclear cells as argued previously above. Thus, in my opinion, the method described in Section 4.9.2 involves the preparation of RNA from isolated nucleated cells which as described above is used to mean mononuclear cells and there is certainly no explicit description of a method involving the extraction of RNA from whole blood in this section or elsewhere in the text" (paragraph 32 of the attached Declaration).

The remaining Examples also demonstrate that the teachings of Ralph et al. are specifically directed solely to using RNA from isolated mononuclear cells, as attested to in Part B of the attached expert declaration. Example 1/Section 5.1 of Ralph et al. discusses using "total cell RNA" to convert to cDNA before beginning PCR (column 71, line 63). As noted above, the "total cell RNA" terminology as used by Ralph et al. refers to RNA from mononuclear cells as noted in section 4.9.1. Example 1/Section 5.1 later describes the preferred methods for isolating the RNA from the isolated mononuclear cell fraction using either the guanidinium thiocyanate method or the STAT-60 method (column 72, paragraph starting at line 63), however as noted above, the method of isolating the RNA free from DNA, polysaccharides and the like is independent from the type of sample that the RNA is being isolated from.

Example 2/Section 5.2 of Ralph et al. discusses the use of one of the RNA fingerprinting methods and notes "for this study, total cell RNA was isolated from buffy coat cells as described above" (column 79, line 15-16). This is in clear reference to the method described in section 4.9.1 which uses the term "buffy coat" to refer to mononuclear cells isolated using Ficoll gradient fractionation via CPT™ Vacutainer™ tubes, and which uses the term "total cell RNA" to refer to RNA extracted from isolated mononuclear cells.

Example 3/Section 5.3 of Ralph et al. describes the method of RNA fingerprinting for analyzing certain genes and Example 4/Section 5.4 describes the sequencing of these genes.

Example 5/Section 5.5 of Ralph et al. describes the use of the arbitrarily primed PCR method of fingerprinting and of relative quantitative RT-PCR to analyze IL-8 gene expression. As noted above, although the term "peripheral blood leukocytes" is used, Applicant notes that this clearly does not refer to leukocytes which have not been fractionated into cell types, but rather refers solely to mononuclear cells.

Example 6/Section 5.6 of Ralph et al. indicates that RNA fingerprinting was performed as described in section 4.12 (column 95, line 36-37). As noted above section 4.12.2 refers to section 4.11.1 for a description of the actual RNA which is utilized for the experiments and

section 4.11.1 refers to isolation of RNA from mononuclear cells isolated as described in section 4.9.1 (column 67 line 12-13).

Example 7 of Ralph et al. describes use of the same procedures which are used with respect to the IL-8 gene, as described in Example 5 of Ralph et al. Thus, Example 7 also refers to mononuclear cells. A reference may only be relied upon for what it would have reasonably suggested to one having ordinary skill the art (Merck & Co. v. Biocraft Laboratories, 874 F.2d 804, 10 USPQ2d 1843 (Fed. Cir.), cert. denied, 493 U.S. 975 (1989)). As demonstrated above, and as demonstrated and attested to in the attached expert declaration, Ralph et al. teaches the use of RNA obtained only from isolated mononuclear cells, and all references throughout Ralph *et al.* are to RNA prepared from mononuclear cells. As such, Ralph et al. can only reasonably suggest to a person skilled in the art methods based on use of mononuclear cells. A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference" Verdegaal Bros. v. Union Oil Co. of California, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987), and as such, the Ralph *et al.* reference does not teach "RNA of blood samples which have not been fractionated into cell types", as required by the claims.

The attached expert declaration of Paul Dobner, Ph.D. dated August 18, 2007, concludes that "Ralph et al does not describe or suggest a method of identifying two or more markers useful for diagnosing a disease, said method comprising using RNA of blood samples which have not been fractionated into cell types, as recited by the instant claims", thus supporting Applicant's position that a person skilled in the art, upon reading Ralph *et al.* would understand that the teachings are related to use of blood samples limited to only those which have been fractionated into cell types.

Nevertheless, in order to more clearly indicate that the RNA levels which are compared in Applicant's claims, in contrast to Ralph *et al.*, are those of blood samples which include all of the types of leukocytes in blood, i.e. of blood samples which include granulocytes in addition to mononuclear cells (T-lymphocytes, B-lymphocytes and monocytes), Applicant has newly added claims 57 and 58, which include the recitation "blood samples which comprise leukocytes which have not been fractionated into cell types". As described above, the recitation "blood samples which comprise leukocytes which have not been fractionated into cell types" finds clear support in the specification at Figure 5C, which shows fractions of different types of leukocytes which

collectively constitute "leukocytes which have not been fractionated into cell types", i.e. granulocytes, T-lymphocytes, B-lymphocytes and monocytes (labeled "G.R.", "CD 3+", "CD19" and "MONO", i.e., respectively). The fact that granulocytes, lymphocytes (T-lymphocytes and B-lymphocytes) and monocytes collectively represent all of the types of leukocytes found in blood is evident in Immunobiology. Garland Publishing. 2001. Fifth Edition. Janeway, Travers, Walport, and Shlomchik, eds. Fig. A.23, of record., which clearly teaches that leukocytes are composed of granulocytes and mononuclear cells, and that the latter are composed of lymphocytes and monocytes. The ordinarily skilled artisan will readily understand, particularly in view of the legend of Figure 5C which recites "*FIG. 5C shows... each fractionated cell from whole blood.*", that data representing each type of blood leukocyte is represented in Figure 5C; that "G.R." is an acronym representing granulocytes; that "CD 3+" refers to CD3+ cells, i.e. T-lymphocytes; that "CD19" refers to CD19+ cells, i.e. B-lymphocytes; that "MONO" is an acronym representing monocytes; and that all lymphocytes are represented in Figure 5C since whole lymphocytes consist of T- and B-lymphocytes. It is well known to the ordinarily skilled artisan that CD3 and CD19 are specific cell surface markers of T-lymphocytes and B-lymphocytes, respectively (refer, for example, to the enclosed Abstract of Casey *et al.*, 1988. A simplified plastic embedding and immunohistologic technique for immunophenotypic analysis of human hematopoietic and lymphoid tissues. Am J Pathol. 131:183-9). Literal support for the term "leukocytes" can be found at paragraph [0005] of the Published Application.

Thus, Applicant respectfully submits that neither US 6,190,857 nor WO 98/24935 (which the Examiner asserts contains a substantially identical disclosure to US 6,190,857) anticipates claims 17, 19, 57 and 58. Reconsideration and withdrawal of the rejections is requested.

35 U.S.C. § 103(a) Rejections – Obviousness

The Examiner has rejected claim 43 as being obvious over WO98/24935 to Ralph et al. or US 6,190,857 to Ralph *et al.* in view of Sharma et al. (WO98/49342). Applicant respectfully traverses the rejections.

In particular, the Examiner refers to teachings which the Examiner contends are taught by Ralph et al. in making the above rejections under 35 U.S.C. § 102 (made in the office action dated July 2, 2007). In making these rejections under 35 U.S.C. § 102, the Examiner asserts that Ralph et al. teaches the use of *"an oligonucleotide of predetermined sequence which are primers specific to... particular transcripts" for detection of marker RNAs of diseases. The Examiner*

claims that "Ralph et al. teach the use of their method to discover disease markers for any disease state that affects the peripheral blood leukocytes, including metastatic or organ defined cancer" citing column 9, line 66 and column 19, line 3 of US 6,190,857 to Ralph *et al., but concedes that Ralph et al. fails to teach that the disease is colorectal cancer.* The Examiner further claims that Sharma et al. teach a method of identifying two or more markers useful for diagnosing a disease by looking for differentially expressed genes in total RNA isolated from whole blood samples, and that Sharma et al. teach that a disease in which their method would be useful is cancer of the bowel. As a result, the Examiner's position is that it would have been prima facie obvious to modify the methods taught by Ralph et al. so as to have screened for markers of colorectal cancer as taught by Sharma et al., making claim 43 obvious.

Applicant respectfully disagrees that claim 43 is rendered obvious by either of the Ralph et al. references in view of Sharma et al. since Sharma et al. clearly and explicitly teaches away from using "sequence based methods", such as RT-PCR using "*an oligonucleotide of predetermined sequence which are primers specific to... particular transcripts*" for identifying disease diagnostic probes, which is the method which the Examiner asserts is taught by the Ralph et al. references in the rejections under 35 U.S.C. § 102 made in the office action dated July 2, 2007. Applicant's position that it would be improper to combine either of the Ralph et al. references with Sharma et al. is clearly in accordance with the Examiner's assertion in the Office Action dated March 8, 2006 (page 27) that "*Sharma et al. specifically teach away from any step used for identifying probes that are diagnostic for disease where the step uses a sequence based method*" and that "*Given this express teaching by Sharma, there would be no motivation to combine the teachings of Sharma et. al with a reference which suggests the use of certain gene specific primers for the amplification and detection of only a subset of differentially expressed genes. The claims are thus free of the teachings of Sharma et al.*". Applicant's position that it would be improper to combine either of the Ralph *et al.* references with Sharma *et al.* due to the latter's counterteachings is further clearly supported by the guidelines at MPEP § 2145(X)(D)(2) and the case law which indicate that it is improper to combine references for purposes of an obviousness rejection, where one reference teaches away from their combination. See, for example, In re Grasselli, 713 F.2d 731, 743, 218 USPQ 769, 779 (Fed. Cir. 1983) "It is improper to combine references where the references teach away from their combination"; Winner International Royalty Corp. v. Wang, 202 F.3d 1340, 53 USPQ2d 1580 (Fed. Cir. 2000) "if

Johnson did in fact teach away from Moore, then that finding alone can defeat Wang's obviousness claim"; and Tec Air, Inc. v. Denso Manufacturing Michigan Inc., 192 F.3d 1353, 52 USPQ2d 1294 (Fed. Cir. 1999) "there is no suggestion to combine, however, if a reference teaches away from its combination with another source".

In addition, Applicant disagrees that claim 43 is rendered obvious by either of the Ralph et al. references in view of Sharma et al. since the Ralph et al. references do not demonstrate, suggest nor in any way teach a method which uses RNA of blood samples which have not been fractionated into cell types, as required by claim 43. As attested to in the attached declaration of expert declarant Paul Dobner, Ph.D., dated August 18, 2007, and as elaborated above in Applicant's response to the present 35 U.S.C. § 102 rejections, a person skilled in the art would not read either of the Ralph et al. references as suggesting a method of identifying two or more markers useful for diagnosing a disease using RNA of blood samples which have not been fractionated into cell types, in accordance with the recitation:

> "*Therefore, Ralph et al does not describe or suggest a method of identifying two or more markers useful for diagnosing a disease, said method comprising using RNA of blood samples which have not been fractionated into cell types, as recited by the instant claims*" (paragraph #34 of the declaration).

Thus, in view of Ralph et al.'s clearly teaching analysis of RNA only from isolated mononuclear cells, Applicant submits that the invention of claim 43, which requires analysis of "blood samples which have not been fractionated into cell types", inherently cannot be arrived at by modifying Ralph et al.'s method so as to analyze, in RNA from an isolated mononuclear fraction of blood, markers of bowel cancer in particular, in view of Sharma et al.'s putative teachings regarding analysis of bowel cancer markers in whole blood. Thus, the combination of Sharma et al. and either of the Ralph et al. references clearly fails to teach or suggest the claim 43 limitation of detecting a presence of a colorectal cancer marker RNA in RNA of blood samples which have not been fractionated into cell types, and as such does not render claim 43 prima facie obvious, in accordance with the following quotation from MPEP § 2143.03:

> To establish prima facie obviousness of a claimed invention, all the claim limitations must be taught or suggested by the prior art. In re Royka, 490 F.2d 981, 180 USPQ 580 (CCPA 1974).

As such, claim 43 is not rendered obvious by the Ralph et al. references in view of Sharma *et al.* In view of the above arguments, Applicant respectfully requests reconsideration and withdrawal of the rejections.

Double patenting rejections

While respectfully disagreeing with the contention that the claims can be rejected under double patenting rejections, Applicant will consider filing a terminal disclaimer should it be necessary upon the indication of allowable claims.

Conclusion

Applicant submits that all claims are allowable as written and respectfully request early favorable action by the Examiner. If the Examiner believes that a telephone conversation with Applicant's attorney/agent would expedite prosecution of this application, the Examiner is cordially invited to call the undersigned attorney/agent of record.

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Respectfully submitted,

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Abstract of: Casey et al., 1988. simplified plastic embedding and immunohistologic technique for immunophenotypic analysis of human hematopoietic and lymphoid tissues. Am J Pathol. 131:183-9.

Article of: Chomczynski and Sacchi, 1987. Anal. Biochem. 162: 156-159.